Optimization of DNA Nanoparticle Mediated Gene Therapy for Inherited Ocular Diseases

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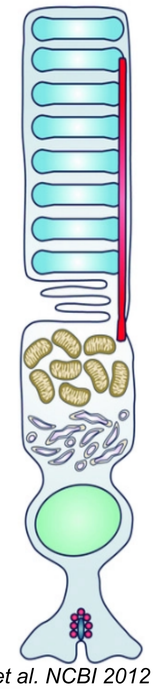
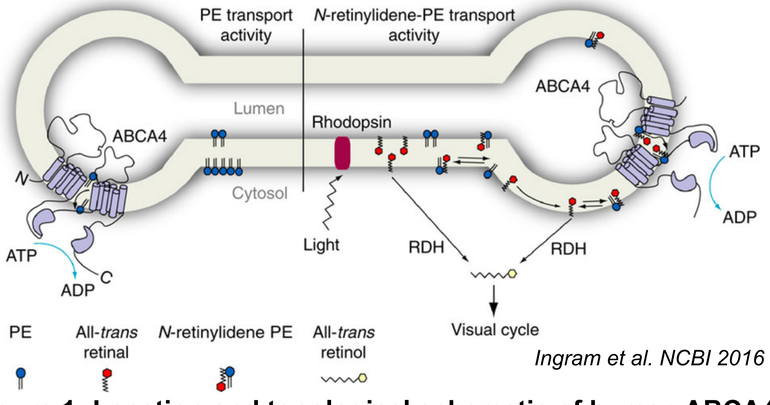
Introduction & Background

Mutations in the photoreceptor-specific flippase, ATP-binding cassette subfamily A member 4 (ABCA4), lead to Stargardt's Macular Degeneration, a blinding disorder. Currently no therapies are available to correct this, however, gene therapy appears to be a possible solution. While viral-mediated methods with adeno-associated viral (AAV) vectors have shown to be successful in gene therapy, they are limited in carrying capacity. Due to the large size of the ABCA4 gene, a different approach is required. The application of compacted DNA nanoparticles (NP) with higher capacity, is an alternative. Besides their larger payload, NPs have demonstrated successful long-term expression in previous gene therapy studies. In this study, DNA NPs containing the correct gene sequence were subretinally and vitreally injected and later analyzed.



Quazi et al. NCBI 2012



Ingram et al. NCBI 2016

Figure 1: Location and topological schematic of human ABCA4.

- ABCA4 located in outer segments of disks in rod and cone photoreceptors (PRs).
- Large gene with cDNA = 6.8 kbp. (genomic DNA ~200 kbp)
- Splitting the gene into asymmetrical N-terminus and C-terminus halves can reduce size of gene and still achieve full expression by interaction between the halves.
- Mutations can lead to Stargardt's Macular Degeneration with prevelence of ~1:10,000.
- NPs used in this study are 8-10nm in diameter and formulated with polyethylene glycol-substituted polylysine (CK30PEG) and either a CMV or MOP promoter.

Hypothesis

Phenotypic rescue of ABCA4 in ABCA4-deficient mice can be achieved via either intravitreal or subretinal injections of compacted DNA nanoparticles.

Methodology

Restriction Enzyme Digestion:

- Previous work in lab used a pEPI-MOP-ABCR vector as a starting point.
- MOP promoter is PR specific for *in-vivo* use.
- For *in-vitro* tests, vector required swapping of retina specific MOP promoter with non-tissue specific CMV promoter.

Plasmid DNA Purification:

- After CMV promoter ligated with full-length, N-half, and C–half, DNA was replicated via colony PCR.
- Colony DNA was extracted, grown in medium, and purified for sequencing and future experiments.
- These experiments allowed for *in-vitro* modifications and optimization.
- Following all *in-vitro* experiments, DNA was assayed for endotoxins, compacted, and injected at set time points.
- Eyes were extracted, sectioned, and panretinal distribution and expression was analyzed. (Supplementary data)

Restriction Enzyme Digestion

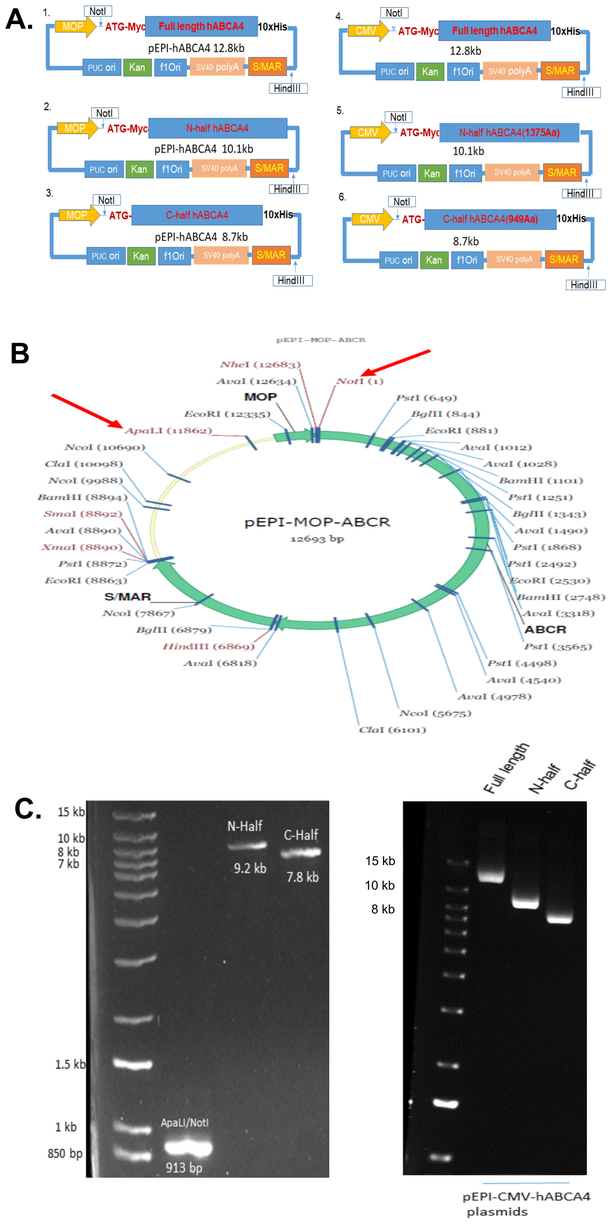


Figure 3: Replacement of MOP promoter with CMV promoter.

A. Map of hABCA4 vector and its encoded genes. A1-A3 are the starting vectors containing MOP promoter, A4-A6 contain CMV promoter after replacement.

B. Restriction enzyme map of full-length pEPI-MOP-ABCR vector. NotI and ApaLI sites were used to release MOP and reused to insert CMV. ApaLI and NotI target sites marked with red arrow.

C. Left: Released ApaLI/NotI fragment containing MOP promoter. Digested N-half vector. Digested C-half vector. The released fragments were then gel purified.

Right: Confirmation of gel purified digested full-length, N-half, and C-half vectors. The fragments were then further gel purified and ligated with CMV fragments.

Plasmid DNA Purificaiton

Results:

Sample Name	Nucleic Acid(ng/uL)	A260/A280	A260/A230
N-Half:2 + CMV	4407.403	1.889	2.34
N-Half:2 + CMV	4386.798	1.902	2.359
C-Half:2 + CMV	3010.898	1.895	2.367
C-Half:2 + CMV	3021.201	1.889	2.35
Full:5 + CMV	3290.428	1.897	2.342
Full:5 + CMV	3295.897	1.899	2.345

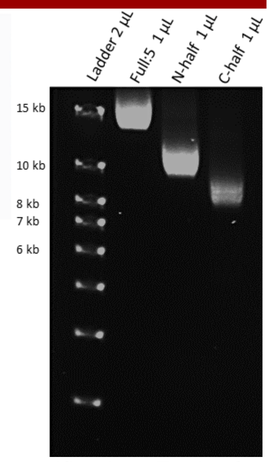


Figure 4: Endotoxin-Free (EF) Plasmid DNA Purification.

Following restriction enzyme digestion and ligation with CMV fragment, the newly synthesized vectors were replicated via colony PCR then purified of endotoxins.

The table above shows the results of the purification. Each of the samples were resuspended in 300 µL EF-H_2O. Ratios A260/A280 and A260/A230 are ratios of DNA:Protein and DNA:impurities, respectively. Samples were then diluted and 1µL was loaded from each.

Summary and Conclusions

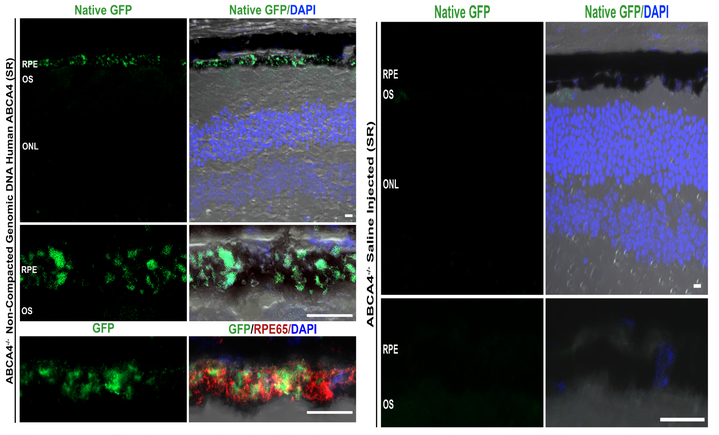


Figure 5: Injected DNA expression (left) and non-injected expression (right).

- GFP expression was detected in RPE layer of P60 eyes injected with non-compacted, full-length pEPI-MOP-ABCA4.
- Expression in RPE layer confirmed by coexpression of RPE65, a RPE specific protein.
- No GFP detected in saline injected eyes.

Notable GFP expression was detected by immunofluorescence in adult mice injected with large ABCA4 constructs. This work suggests that the vectors may be excellent candidates for delivery of therapeutic genes to patients affected by inherited retinal diseases.

Acknowledgments

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